

We Claim:

1. A method for preparing an evolved microorganism comprising the steps of:
 - a. culturing a microorganism comprising at least one heterologous mutator gene for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type, and
 - b. restoring said evolved microorganism to a wild type mutation rate.
- 10 2. The method of Claim 1 wherein said microorganism further comprises at least one introduced nucleic acid encoding a heterologous protein.
- 15 3. The method of Claim 2 wherein said heterologous protein(s) includes hormones, enzymes and growth factors.
- 15 4. The method of Claim 3 wherein said heterologous protein is an enzyme.
- 20 5. The method of Claim 4 wherein said enzyme includes hydrolases, such as protease, esterase, lipase, phenol oxidase, permease, amylase, pullulanase, cellulase, glucose isomerase, laccase and protein disulfide isomerase.
- 25 6. The method of Claim 1 wherein said microorganism further comprises introduced nucleic acid encoding at least one enzyme necessary for an enzymatic pathway.
- 25 7. The method of Claim 6 wherein said enzyme is a reductase or a dehydrogenase and said enzymatic pathway is for the production of ascorbic acid or ascorbic acid intermediates.
- 30 8. The method of Claim 6 wherein said enzyme is glycerol dehydratase or 1,3-propanediol dehydrogenase and said enzymatic pathway is for the production of 1,3 propanediol, 1,3 propanediol precursors or 1,3 propanediol derivatives.

9. The method of Claim 6 wherein said enzyme is glycerol-3-phosphate dehydrogenase or glycerol-3-phosphate phosphatase and said pathway is for the production of glycerol and glycerol derivatives.

5 10. The method of Claim 6 wherein said enzymatic pathway is for the production of amino acids or dyes.

11. The method of Claim 1 wherein said microorganism is cultured for between about 20 to about 100 doublings.

10 12. The method of Claim 1 wherein said microorganism is cultured for between about 100 to about 500 doublings.

13. The method of Claim 1 wherein said microorganism is cultured for 15 between about 500 to about 2000 doublings.

14. The method of Claim 1 wherein said microorganism is cultured for greater than 2000 doublings.

20 15. The method of Claim 1 wherein said evolved microorganism comprises from about 3 to about 1000 selected mutations.

16. The method of Claim 1 wherein said evolved microorganism further comprises from about 20 to about 100,000 neutral mutations

25 17. The method of Claim 1 wherein said evolved microorganism comprises about 3 to about 1000 selected mutations in about 3 to about 500 genes.

18. The method of Claim 17 wherein said mutations are non-specific.

30 19. The method of Claim 17 wherein said mutations are specific.

20. The method of Claim 1 wherein said mutator gene generates a mutation rate of at least about 5 fold to about 10,000 fold relative to wild type.

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21. The method of Claim 1 wherein said mutator gene generates a mutation rate of at least about 5 fold to about 1000 fold.

22. The method of Claim 1 wherein said mutator gene generates a mutation rate of about 5 fold to about 1000 fold over wild type.

23. The method of Claim 1 wherein said microorganism comprises a plasmid comprising the heterologous mutator gene and said step of restoring said evolved microorganism to a wild type mutation rate comprises curing the evolved microorganism of said plasmid.

24. The method of Claim 23 wherein said plasmid comprises a temperature sensitive origin of replication.

25. The method of Claim 1 wherein said microorganism comprises at least one copy of the mutator gene in the chromosome and said step of restoring said evolved microorganism to wild type mutation rate comprise excision of said mutator gene.

26. The method of Claim 1 wherein said mutator gene comprises mutD, mutT, mutY, mutM, mutH, mutL, mutS or mutU mutations or homologues thereof.

27. The method of Claim 26 wherein said mutator gene comprises mutD having mutations shown in Table I.

28. The method of Claim 1 wherein said conditions suitable for selection comprise culturing said microorganism in the presence of at least one organic solvent.

29. The method of Claim 28 wherein said organic solvent includes alcohols, diols, hydrocarbon, mineral oil, mineral oil derived products, halogenated compounds and aromatic compounds.

30. The method of Claim 1 wherein said conditions suitable for selection comprise culturing said microorganism in the presence of elevated temperature.

31. The method of Claim 30 wherein said elevated temperature is about 42° C to about 48° C.

5 32. The method of Claim 1 wherein said conditions suitable for selection comprise culturing said microorganism in the presence of high salt.

10 33. The method of Claim 1 wherein said microorganism includes Gram-positive or a Gram-negative microorganism, fungus, yeast or eucaryotic.

15 34. The method of Claim 33 wherein said microorganism is an *Enterobacteriaceae*.

15 35. The method of Claim 34 wherein said microorganism is an *Eschericia*.

15 36. The method of Claim 35 wherein said microorganism is *E.coli*.

20 37. The method of Claim 35 wherein said microorganism is *E.blatte*.

20 38. The method of Claim 1 wherein said evolved microorganism is *E.coli* having ATCC accession number _____.

25 39. The method of Claim 1 wherein said evolved microorganism is *E.blattae* having ATCC accession number _____.

25 40. An expression vector comprising a mutator gene.

30 41. The expression vector of Claim 40 wherein said mutator gene is a mutated MutD.

30 42. The expression vector of Claim 40 wherein said mutated MutD has the mutations as shown in Table I.

30 43. A host cell comprising the expression vector of Claim 40.

44. The host cell of Claim 43 that is a Gram-positive or Gram-negative microorganism.

5 45. The host cell of Claim 44 that is an Enterobacteriaceae.

46. The isolated E. blattae microorganism deposited with the ATCC and having accession number.

10 47. The isolated E.coli microorganism deposited with the ATCC and having accession number.

48. A method for preparing an evolved microorganism comprising the steps

of:

15 a. mutating a DNA repair gene in a microorganism to obtain a mutated strain,
b. culturing the mutated strain for at least 20 doublings under conditions suitable for selection of an evolved strain, wherein said mutated strain generates a mutation rate of at least 5-100,000 fold relative to the wild-type microorganism, and
20 c. restoring the naturally occurring DNA repair gene in said evolved microorganism.